

## Quality control of heparins

**Application**

**Heinz Goetz**

Heparins are mucopolysaccharides formed by the reaction of D-glucoseamines and D-glucuronic acid. They are widely used as anticoagulants to prevent thrombolysis in case of hyperlipidemia, arteriosclerosis, blood transfusions and operations. Heparins have molecular weights in the range of 4,000 to 35,000 daltons. Low molecular weight heparins are a class of compounds derived from standard heparin with somewhat different properties. They contain molecules from

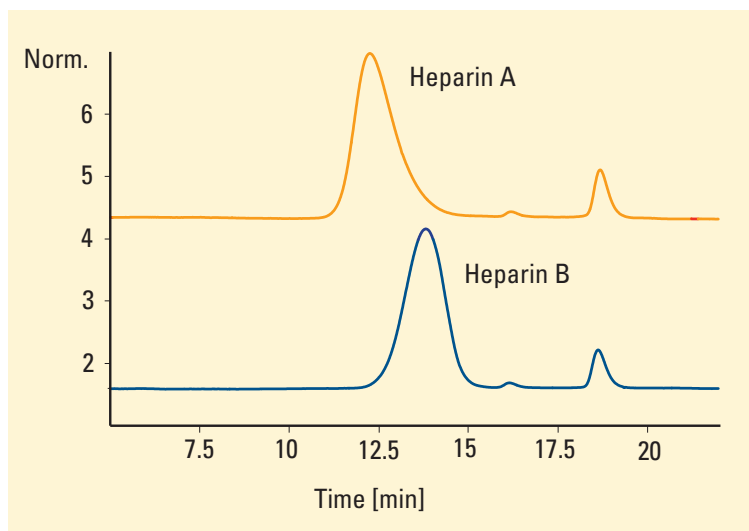


Figure 1  
Overlay of chromatograms of a high and a low molecular weight heparin

### Conditions

#### Sample preparation

Samples were dissolved in the mobile phase and filtered (0.45  $\mu\text{m}$ )

#### Column

3  $\times$  PL aquagel-OH 30 in series, 7.5  $\times$  300 mm, 8  $\mu\text{m}$  (Agilent p/n 79911GF-083)

#### Mobile phase

0.2 M  $\text{NaNO}_3$ ,  $\text{NaH}_2\text{PO}_4$ , pH 7

#### Flow rate

1 mL/min

#### Column compartment temperature

25  $^\circ\text{C}$

#### Injection volume

100  $\mu\text{l}$

#### Detector

Refractive index detector



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the lower end of the molecular weight range and to date clinical studies in humans have shown advantages of low molecular weight heparins over standard heparins. As the molecular weight clearly has a strong effect on the usage of heparin in clinical studies, characterization is vital in both research and development and quality control.

Figure 1 shows typical raw data chromatograms for two lots of heparin. By calibrating the column set using polysaccharide standards, it is possible to simultaneously produce molecular weight distributions and averages for the two materials. The data below shows that sample A is of high molecular weight and sample B of low molecular weight.

Sample A:  $M_n$ : 22500     $M_w$  28200  
Sample B:  $M_n$  9350     $M_w$  10770

## HPLC performance

RSD of  $M_w$     < 1.5%  
RSD of  $M_n$     < 5%

## Equipment

### Agilent 1100 Series GPC-SEC system

consisting of

- vacuum degasser for efficient degassing of the mobile phase
- isocratic pump with large solvent cabinet
- autosampler with single valve design
- thermostatted column compartment for precise column temperatures
- refractive index detector with automatic recycle valve
- ChemStation Plus with GPC-SEC data analysis software

Heinz Goetz is an application chemist at Agilent Technologies, Waldbronn, Germany

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